I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on December 17, 2010.

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 AND UNDER 37 CFR 1.323
Docket No. SER.107

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Mara Rossi, Thierry Ziegler, Laure Valognes
Issued	:	October 26, 2010
Patent No.	:	7,820,800
Serial No.	:	10/576,372
Conf. No.	:	9428
For	:	Process for the Purification of IL-18 Binding Protein

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE) AND
UNDER 37 CFR 1.323 (APPLICANT MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appeared or should have appeared.

Patent Reads:	**Application Reads:**
Column 6, line 33:	Page 9, line 3:
"at. pH"	--at pH--

Column 11, lines 38-39:

"thereof. "Functional derivatives" as used"

Page 17, lines 5-6:

--thereof.
"Functional derivatives" as used--

Patent Reads:

Column 14, line 67:

"is not any"

Application Should Read:

Page 22, line 7:

--is not in any--

Column 15, line 12:

"meaning an range"

Page 22, line 14:

--meaning and range--

Column 15, lines 32-33:

"adding few drops"

Page 22, line 26:

--adding a few drops--

Column 16, lines 18-19:

"adding few drops"

Page 24, line 16:

--adding a few drops--

Patent Reads:

Column 16, lines 21-22:

"with 5-4 BV"

Application Reads:

Page 24, line 18:

--with 5-6 BV--

Patent Reads:

Column 16, lines 29-30:

"checked and, and washing"

Application Should Read:

Page 24, line 23:

--checked and washing--

Patent Reads:

Column 17, line 4:

"-MEP"

Application Reads:

Page 25, line 27:

--MEP--

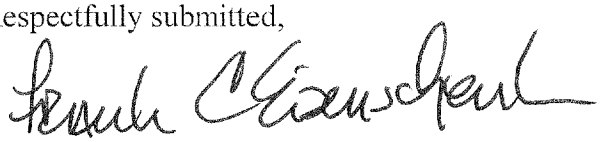
Patent Reads:	**Application Should Read:**
Column 18, line 64:	Page 29, line 8:
"(2-N-Morpholino)ethanesulfonic acid)"	--(2-[N-Morpholino]ethanesulfonic acid)--
Column 19, line 57:	Page 30, line 25:
"2 Gillson"	--2 Gilson--
Patent Reads:	**Application Reads:**
Column 21, line 56:	Page 34, line 11:
"34 BV"	--3-4 BV--
Patent Reads:	**Application Should Read:**
Column 21, lines 57-58:	Page 34, lines 11-12:
"the column was rinsed the column with"	--the column was rinsed with--.

A true and correct copy of pages 9, 17, 24, 25 and 34 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The fee of $100.00 was paid at the time this Request was filed. The Commissioner is also authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/ps

Attachments: Copy of pages 9, 17, 24, 25 and 34 of the specification

Step (b) is preferably carried out on a MEP (4-mercaptoethylpyridine derivative) column, such as MEP HyperCel® (LifeSciences). Binding of IL-18BP is carried out preferably at pH 6.1 ± 0.1, e.g. in PBS 1X + 1 NaCl having this pH. Elution is carried out preferably at pH 8.4 ± 0.1, e.g. in with 20 mM phosphate buffer plus 35% propylene glycol, the mixture having pH 8.4 ± 0.1.

Step (c) is preferably carried out on a carboxymethyl-sepharose (CM) column. This is a step in which the flow-through is collected for further purification. This step is based on the fact that under specific circumstances relating e.g. to salt and pH conditions, IL-18BP does not bind to the resin, while impurities (e.g. host cell proteins, serum-derived proteins) that is used for bind to it. Preferably, step (c) is carried out at pH 6.0±0.2, for example in the presence of 1 mM MES (N-morpholinoethanesulfonic acid).

Step (d) is preferably carried out on a phenyl sepharose column, such as a Phenyl-Sepahrose Fast Flow column. Preferably, binding of IL-18BP is carried out at about pH 9.1 ± 0.2, e.g. in 50 mM sodium borate and 0.9 M ammonium sulphate or 0.10 M ammonium sulfate having this pH. The elution from the phenyl-sepharose column is preferably carried out at pH 9.1 ± 0.2 in the presence of an elevated salt concentration, such as in 50 mM sodium borate 9.1 ± 0.2, 0.15M ammonium sulphate having this pH.

Step (e) is preferably carried out on a Source 30 RPC column. Binding of IL-18BP to the column material is preferably carried out at pH 9.1 ± 0.2, e.g. in 50 mM sodium borate buffer. Elution is preferably carried out using a gradient, IL-18BP eluting around 28-32% of 0,1% trifluoroacetic acid (TFA) in acetonitrile.

It is understood that the conditions described above in connection with steps (a) to (e) of the purification may also be applied when carrying out single steps of the invention, or (sub-)combinations of steps.

In a further preferred embodiment of the present purification process, one or more ultrafiltration steps are performed. Ultrafiltration is useful for removal of small molecular weight components in the eluates resulting from previous chromatrographic steps. This ultrafiltration allows removing organic solvent, TFA and salts from the previous step, to equilibrate the IL-18BP in the bulk buffer and to concentrate the molecule to the desired concentration. Such ultrafiltration may e.g. be performed on ultrafiltration media excluding components having molecular weights below 5 kDa.

The term "fused protein" refers to a polypeptide comprising an IL-18BP, or a viral IL-18BP, or a mutein or fragment thereof, fused with another protein, which, e.g., has an extended residence time in body fluids. An IL-18BP or a viral IL-18BP, may thus be fused to another protein, polypeptide or the like, e.g., an immunoglobulin or a fragment thereof.

"Functional derivatives" as used herein cover derivatives of IL-18BPs or a viral IL-18BP, and their muteins and fused proteins, which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein which is substantially similar to the activity of IL-18BP, or viral IL-18BPs, and do not confer toxic properties on compositions containing it.

These derivatives may, for example, include polyethylene glycol side-chains, which may mask antigenic sites and extend the residence of an IL-18BP or a viral IL-18BP in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fractions" of an IL-18BP, or a viral IL-18BP, muteins and fused proteins, the present invention covers any fragment or precursors of the polypeptide chain of the protein molecule alone or together with associated molecules or residues linked thereto, e.g., sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fraction has substantially similar activity to IL-18BP.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of IL-18 inhibitor molecule, or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric

Buffers and solutions

- Metal charge solution: 0.2 M Copper Sulphate
- Acidified water
- Equilibration buffer: 50 mM Sodium phosphate pH 8.5 ± 0.1, 0.5 M NaCl.
- Elution buffer: 0.075 M Ammonium Acetate pH 9.0 ±0.1
- Regeneration solution: 20 mM sodium phosphate pH 5.8 ± 0.3, 0.5 M NaCl, 50 mM EDTA
- Sanitizing solution: 0.5 M NaOH

The column was packed with Chelating Sepharose Fast Flow resin following the manufacturer's instructions. For sanitization, the column was flushed with at least 3 BV of NaOH 0.5 M, incubated for 1 hour at room temperature, and then the column was rinsed with 3 BV of purified water.

220-300 mg of concentrated r-hIL-18BP obtained from capture step reported above under (1) were thawed and adjusted to pH to 8.5 ± 0.1, conductivity 50±5 mS/cm by adding few drops of 35% ortho-phosphoric acid (H_3PO_4) and solid NaCl in the amount corresponding to about 0.35M.

The chromatographic column was first flushed with 5-6 BV (bed volumes) of acidified water until pH was <4.5. Then, the column was flushed with 3 BV of 0.2 M copper sulphate and 4 BV of acidified water until absorbance reached the baseline.

Then, the column was equilibrated by flushing 6 or more BV of equilibration buffer, 50 mM Sodium phosphate pH 8.5 ± 0.1, 0.5 M NaCl, conductivity 50 ± 5 mS/cm through the column. The pH and conductivity were checked and, and washing was continued if the parameters of the column's effluent are out of target values, i.e. pH 8.5±0.1, conductivity 50±5 mS/cm.

The starting material, i.e. post capture r-hIL-18BP prepared as above, was then loaded onto the column. After completion of sample loading, the column was flushed with 5-10 BV of equilibration buffer. These factions were discarded, since they contained only cell culture impurities.

Elution was started with 0.075 M ammonium acetate pH 9.0 ±0.1, conductivity 7.6±0.5 mS/cm. r-hIL-18BP started eluting as a main peak after about 0.5 BV from the start.

3-5 BV of the main peak were collected, the main peak starting when the on-line OD steeply increased. This fraction contained semi-purified r-hIL-18BP.

After completion of the elution, the column was flushed with 3-5 BV of regeneration buffer containing EDTA. The sampled fractions contained copper displaced from the resin, as well as cell culture impurities.

For sanitization, the column was flushed with at least 3 BV of NaOH 0.5 M, incubated for 1 hour, and then the column was rinsed with 3 BV of purified water. The column was then flushed with at least 3 BV of storage solution, 10 mM NaOH and is stored it at room temperature until the next cycle.

2.2. Step (b): HIC/IEC on MEP Hypercel

This step is carried out on MEP resin, a hydrophobic charge-induction chromatography resin, which is a mixture between hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC).

Equipment

- Chromatographic column: XK1.6 x 20cm (Amersham Biosciences);
- UV monitor (optical path Length 2.5 mm) equipped with a two channel recorder (Amersham Biosciences or equivalent);
- Peristaltic pump (Minipuls Gilson or equivalent);
- UV Spectrophotometer (Shimadzu or equivalent);
- pH meter (Metrohm or equivalent);
- Conductometer (Metrohm or equivalent);
- Balance (MettlerToledo or equivalent).

Materials

- Post IMAC r-hIL-18BP;
- MEP HyperCel® resin (BioSepra – Cypergen Biosystems);
- Sodium Hydroxide pellets – (Merck) ;
- Potassium Chloride-(Merck);
- Ethylenediaminetetracetic acid – EDTA- (Fluka);
- Sodium chloride – NaCl - Merck;
- Purified water (Modulab or equivalent);
- Di-Sodium hydrogen phosphate epta-hydrate – Merck;

r-hIL-18BP started to elute at around 28-32% of solution B (see above in bold) and it was completely eluted within 60 minutes (35%B). The eluate was immediately brought to pH 8,0 ± 0,5 by dilution 1:2 with 50 M sodium borate pH 9.1±0.2 and conductivity 5 ± 2mS/cm.

This fraction contains purified r-hIL-18BP.

Column regeneration was carried out at the end of the elution gradient. The regeneration fraction was collected according to the absorbance profile. This fraction contains residues of cell culture impurities and aggregates forms of IL-18BP.

For sanitization, the column was rinsed with 2-3 BV of water, flushed with at least 3-4 BV of NaOH and then the flow stopped for 1 hour. After that, the column was rinsed the column with 3-4 BV of purified water.

The column was flushed with at least 3 BV of storage solution, and stored until the next cycle.

3. Summary of clearance of host cell proteins from the IL-18BP preparation

The amount of host cell proteins was measured by ELISA using a polyclonal antiserum that was raised in rabbit against CHO cell derived contaminants present in serum-free cell culture medium. The amount of host cell proteins is expressed as ppm (parts per million) of contaminating proteins in relation to purified IL-18BP. The amount of IL-18BP was measured by determination of the optical density (OD) at 280 nm (molar extinction coefficient ε= 1.26) in the purified preparation of IL-18BP, i.e. after the final purification step by Reverse Phase chromatography.

This analysis was carried out in the frame of three independent experiments.

Table V: Clearance of host cell proteins

Run	Start IMAC	Post IMAC	Post MEP	Post CM
1	528966 ppm	470600 ppm	68400 ppm	> 4000 ppm
2	475322 ppm	366800 ppm	49800 ppm	558 ppm
3	230400 ppm	236200 ppm	89100 ppm	641 ppm

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,820,800

APPLICATION NO.: 10/576,372

DATED : October 26, 2010

INVENTORS : Mara Rossi, Thierry Ziegler, Laure Valognes

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 6,
Line 33, "at. pH" should read --at pH--.

Column 11,
Lines 38-39, "thereof. "Functional derivatives" as used" should read
--thereof.
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Lines 21-22, "with 5-4 BV" should read --with 5-6 BV--.
Lines 29-30, "checked and, and washing" should read --checked and washing--.

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Column 17,
Line 4, "-MEP" should read --MEP--.

Column 18,
Line 64, "(2-N-Morpholino)ethanesulfonic acid)" should read
--(2-[N-Morpholino]ethanesulfonic acid)--.

Column 19,
Line 57, "2 Gillson" should read --2 Gilson--.

Column 21,
Line 56, "34 BV" should read --3-4 BV--.
Lines 57-58, "the column was rinsed the column with" should read --the column was rinsed with--.

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